Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Complete Listing of All Claims, 37 C,F.R. § 1.121(c)

1. (Original) A compound of formula (XII)

wherein R^7 is H, alkyl, heteroalkyl, aryl, or $-CH_2-C_6H_4OR^{14}$; R^8 is H, alkyl, heteroalkyl, or aryl; R^9 is H, alkyl, heteroalkyl, aryl, or $-C_6H_4OR^{15}$; R^{10} is -H, $-CH_3$, or $-CH(CH_3)_2$; and

R¹¹, R¹⁴, and R¹⁵ are independently enzyme-removable groups; with the proviso that R¹¹, R¹⁴, and R¹⁵ are not all acetyl groups.

- 2. (Original) The compound of claim 1, wherein $R^{7} \text{ is -CH}_{2}\text{-}C_{6}\text{H}_{5}, \text{ naphthyl, -CH}_{2}\text{-}C_{6}\text{H}_{4}\text{OH, -CH}_{2}\text{-}C_{6}\text{H}_{4}\text{F, or -CH}_{2}\text{-}} \\ C_{6}\text{H}_{4}\text{OR}^{14}; \\ R^{8} \text{ is -CH}_{2}\text{C}_{6}\text{H}_{5}, \text{-CH}_{2}\text{C}_{6}\text{H}_{11}, \text{-CH}_{2}\text{C}_{5}\text{H}_{9}, \text{ or -(CH}_{2})_{3}\text{NHC}(=\text{NH})\text{NH}_{2}; \text{ and }} \\ R^{9} \text{ is phenyl, indolyl, -C}_{6}\text{H}_{4}\text{OH, -C}_{6}\text{H}_{4}\text{NH}_{2}, \text{-C}_{6}\text{H}_{4}\text{F, or -C}_{6}\text{H}_{4}\text{OR}^{15}.}$
- 3. (Currently Amended) The compound of claim 1, wherein <u>-OR¹¹, -OR¹⁴, and -OR¹⁵</u> R¹⁴, and R¹⁵ are <u>each independently</u> esters.
 - 4. (Original) The compound of claim 1, wherein R¹¹ is acetyl; and

R¹⁴ and R¹⁵ are independently butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

(Original) The compound of claim 1, wherein
 R¹¹ is butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl; and

R¹⁴ and R¹⁵ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

6. (Original) A compound of formula (XII)

wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

 R^{10} is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are independently enzyme-removable groups; and wherein the concentration of the compound in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C is reduced by less than 50% after 45 minutes.

7. (Original) The compound of claim 6, wherein R^7 is $-CH_2-C_6H_5$, naphthyl, $-CH_2-C_6H_4OH$, $-CH_2-C_6H_4F$, or $-CH_2-C_6H_4OR^{14}$; R^8 is $-CH_2C_6H_5$, $-CH_2C_6H_{11}$, $-CH_2C_5H_9$, or $-(CH_2)_3NHC(=NH)NH_2$; and R^9 is phenyl, indolyl, $-C_6H_4OH$, $-C_6H_4NH_2$, $-C_6H_4F$, or $-C_6H_4OR^{15}$.

- 8. (Original) The compound of claim 6, wherein R¹¹, R¹⁴, and R¹⁵ are esters.
- 9. (Original) The compound of claim 6, wherein R¹¹, R¹⁴, and R¹⁵ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
 - 10. (Original) A compound of formula (XII)

R⁸ is H, alkyl, heteroalkyl, or aryl;

R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

 R^{10} is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are independently enzyme-removable groups; and wherein the removal of at least one enzyme-removable group provides a parent compound; and

wherein the time necessary for the concentration of the compound in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50% is greater than the time necessary for the concentration of the parent compound in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50%.

- 11. (Original) The compound of claim 10, wherein the removal of at least two enzyme-removable groups provides the parent compound.
- 12. (Original) The compound of claim 10, wherein the removal of all enzyme-removable groups provides the parent compound.

- 13. (Original) The compound of claim 10, wherein $R^{7} \text{ is -CH}_{2}\text{-}C_{6}\text{H}_{5}, \text{ naphthyl, -CH}_{2}\text{-}C_{6}\text{H}_{4}\text{OH, -CH}_{2}\text{-}C_{6}\text{H}_{4}\text{F, or -CH}_{2}\text{-}} \\ C_{6}\text{H}_{4}\text{OR}^{14}; \\ R^{8} \text{ is -CH}_{2}\text{C}_{6}\text{H}_{5}, \text{-CH}_{2}\text{C}_{6}\text{H}_{11}, \text{-CH}_{2}\text{C}_{5}\text{H}_{9}, \text{ or -(CH}_{2})_{3}\text{NHC}(=\text{NH})\text{NH}_{2}; \text{ and }} \\ R^{9} \text{ is phenyl, indolyl, -C}_{6}\text{H}_{4}\text{OH, -C}_{6}\text{H}_{4}\text{NH}_{2}, \text{-C}_{6}\text{H}_{4}\text{F, or -C}_{6}\text{H}_{4}\text{OR}^{15}.}$
- 14. (Currently amended) The compound of claim 10, wherein $\underline{-OR^{11}}$, $\underline{-OR^{14}}$, and $\underline{-OR^{15}}$ R^{14} , R^{14} , and R^{15} are each independently esters.
- 15. (Original) The compound of claim 10, wherein R¹¹, R¹⁴, and R¹⁵ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
 - 16. (Original) A compound of formula (XIII) or (XIV)

$$R^{11}O$$
 $R^{11}O$
 R^{13}
 R^{12}
 $R^{11}O$
 R^{7}
 R^{10}
 R^{7}
 R^{10}
 R^{7}
 R^{10}
 R

R⁸ is H, alkyl, heteroalkyl, or aryl;
R¹² and R¹³ are independently -H, -OH, alkyl, heteroalkyl, aryl, or -OR¹⁶;
n is 0, 1, or 2; and
R¹¹, R¹⁴, and R¹⁶ are independently enzyme-removable groups.

- 17. (Original) The compound of claim 16, wherein $R^7 \text{ is -CH}_2\text{-C}_6\text{H}_5, \text{ naphthyl, -CH}_2\text{-C}_6\text{H}_4\text{OH, -CH}_2\text{-C}_6\text{H}_4\text{F, or -CH}_2\text{-}} \\ C_6\text{H}_4\text{OR}^{14}; \text{ and} \\ R^8 \text{ is -CH}_2\text{C}_6\text{H}_5, \text{-CH}_2\text{C}_6\text{H}_{11}, \text{-CH}_2\text{C}_5\text{H}_9, \text{ or -(CH}_2)_3\text{NHC}(=\text{NH})\text{NH}_2.} \\$
- 18. (Currently amended) The compound of claim 16, wherein <u>-OR¹¹, -OR¹⁴, and -OR¹⁵ R¹⁴, and R¹⁶ are <u>each indepedently</u> esters.</u>
- 19. (Original) The compound of claim 16, wherein R¹¹, R¹⁴, and R¹⁶ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
 - 20. (Original) The compound of claim 16, wherein n is 1.
 - 21. (Original) A composition, comprising: the compound of claim 1 in solution.
- 22. (Original) The composition of claim 21, wherein the solution is an aqueous solution.
- 23. (Original) The composition of claim 21, wherein the solution comprises DMSO or alcohol.
 - 24. (Original) A composition, comprising: the compound of claim 6, in solution.
- 25. (Original) The composition of claim 24, wherein the solution is an aqueous solution.

- 26. (Original) The composition of claim 24, wherein the solution comprises DMSO or alcohol.
 - 27. (Original) A composition, comprising: the compound of claim 10, in solution.
- 28. (Original) The composition of claim 27, wherein the solution is an aqueous solution.
- 29. (Original) The composition of claim 27, wherein the solution comprises DMSO or alcohol.
 - 30. (Original) A composition, comprising: the compound of claim 16, in solution.
- 31. (Original) The composition of claim 30, wherein the solution is an aqueous solution.
- 32. (Original) The composition of claim 30, wherein the solution comprises DMSO or alcohol.
- 33. (Currently amended) A protected luminophore, which is a modified coelenterazine that includes an imidazolone oxygen; wherein the enol group has been converted to imidazolone oxygen is included in an ester or an ether comprising an enzyme-removable group;

wherein subsequent the removal of said enzyme-removable group providing provides a parent coelenterazine; and

wherein the time necessary for the concentration of the modified coelenterazine in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50% is greater than the time necessary for the concentration of the parent coelenterazine in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50%.

34. (Withdrawn) A kit, comprising: a protected luminophore; and

a luminogenic protein.

- 35. (Withdrawn) The kit of claim 34, further comprising a deprotecting enzyme separate from the luminophore.
- 36. (Withdrawn) The kit of claim 34, wherein the protected luminophore and the luminogenic protein are in separate containers.
- 37. (Withdrawn) The kit of claim 34, wherein the protected luminophore and the luminogenic protein are in the same container.
- 38. (Withdrawn) A kit, comprising:
 a protected luminophore; and
 a deprotecting enzyme;
 wherein the luminophore and the deprotecting enzyme are in separate containers.
- 39. (Withdrawn) A method of measuring the enzymatic activity of a luminogenic protein comprising:

contacting a luminogenic protein, a deprotecting enzyme, and a protected luminophore in solution to form a composition; and detecting light produced from the composition.

- 40. (Withdrawn) The method of claim 39, wherein the luminogenic protein is *Renilla* luciferase.
- 41. (Withdrawn) The method of claim 39, wherein the protected luminophore is a compound of formula (XII)

wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴; R⁸ is H, alkyl, heteroalkyl, or aryl; R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵; R¹⁰ is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are independently enzyme-removable groups.

- $\begin{array}{lll} & \mbox{42.} & \mbox{(Withdrawn) The method of claim 41, wherein} \\ & \mbox{R^7 is $-CH_2$-C_6H_5, naphthyl, $-CH_2$-C_6H_4$OH, $-CH_2$-C_6H_4$F, or $-CH_2$-C_6H_4$OR14; \\ & \mbox{$R^8$ is $-CH_2$C$_6$H$_5, $-CH_2$C$_6$H$_{11}, $-CH_2$C$_5$H$_9, or $-(CH_2)$_3$NHC(=NH)NH$_2; and R^9 is phenyl, indolyl, $-C$_6H_4$OH, $-C$_6H_4NH_2, $-C$_6H_4$F, or $-C$_6H_4$OR15. \\ \end{array}$
- 43. (Withdrawn) The method of claim 41, wherein R¹¹, R¹⁴, and R¹⁵ are esters.
- 44. (Withdrawn) The method of claim 41, wherein R¹¹, R¹⁴, and R¹⁵ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
- 45. (Withdrawn) The method of claim 39, wherein the protected luminophore is a compound of formula (XIII) or (XIV)

$$R^{11}O$$
 R^{12}
 $R^{11}O$
 R^{13}
 R^{12}
 $R^{11}O$
 $R^{11}O$
 $R^{11}O$
 R^{12}
 $R^{11}O$
 $R^{11}O$
 R^{12}
 R^{12}
 R^{13}
 R^{12}
 R^{12}
 R^{13}
 R^{12}
 R^{13}
 R^{12}
 R^{13}
 R^{12}
 R^{13}

R⁸ is H, alkyl, heteroalkyl, or aryl;

R¹² and R¹³ are independently -H, -OH, alkyl, heteroalkyl, aryl, or -OR¹⁶; n is 0, 1, or 2; and

R¹¹, R¹⁴, and R¹⁶ are independently enzyme-removable groups.

46. (Withdrawn) The method of claim 45, wherein $R^7 \text{ is -CH}_2\text{-C}_6H_5, \text{ naphthyl, -CH}_2\text{-C}_6H_4OH, -CH}_2\text{-C}_6H_4F, \text{ or -CH}_2\text{-} \\ C_6H_4OR^{14}; \text{ and} \\ R^8 \text{ is -CH}_2C_6H_5, -CH_2C_6H_{11}, -CH}_2C_5H_9, \text{ or -(CH}_2)_3NHC(=NH)NH}_2.$

47. (Withdrawn) The method of claim 45, wherein R¹¹, R¹⁴, and R¹⁶ are esters.

- 48. (Withdrawn) The method of claim 45, wherein R¹¹, R¹⁴, and R¹⁶ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
 - 49. (Withdrawn) The method of claim 45, wherein n is 1.
- 50. (Withdrawn) The method of claim 39, wherein the composition comprises a cell.
- 51. (Withdrawn) The method of claim 39, wherein the composition comprises a cell which contains the deprotecting enzyme.
- 52. (Withdrawn) The method of claim 51, wherein detecting light produced from the composition indicates the location of the deprotecting enzyme in a cell.
- 53. (Withdrawn) The method of claim 39, wherein the composition comprises a cell lysate.
- 54. (Withdrawn) The method of claim 39, wherein the deprotecting enzyme is an esterase.
- 55. (Withdrawn) The method of claim 39, wherein the solution is an aqueous solution.
- 56. (Withdrawn) The method of claim 39, wherein the solution comprises DMSO.
- 57. (Withdrawn) The method of claim 39, wherein the protected luminophore is a modified coelenterazine;

wherein the enol group has been converted to an ester or an ether comprising an enzyme-removable group.

58. (Withdrawn) A method of generating luminescence in a living cell comprising a luciferase, the method comprising:

contacting the cell in solution with a protected luminophore.

59. (Withdrawn) The method of claim 58, wherein the protected luminophore is a modified coelenterazine;

wherein the enol group has been converted to an ester or an ether comprising an enzyme-removable group.

60. (Withdrawn) The method of claim 58, wherein the protected luminophore is a compound of formula (XII)

wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

 R^{10} is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are independently enzyme-removable groups.

61. (Withdrawn) The method of claim 58, wherein the protected luminophore is a compound of formula (XIII) or (XIV)

$$\mathbb{R}^{11}$$
 \mathbb{R}^{7} \mathbb{R}^{8} \mathbb{R}^{12} \mathbb{R}^{12} \mathbb{R}^{11} \mathbb{R}^{12} \mathbb{R}^{11}

R⁸ is H, alkyl, heteroalkyl, or aryl;

 R^{12} and R^{13} are independently -H, -OH, alkyl, heteroalkyl, aryl, or -OR 16 ;

n is 0, 1, or 2; and

R¹¹, R¹⁴, and R¹⁶ are independently enzyme-removable groups.

62. (Withdrawn) A method of measuring the enzymatic activity of a nonluminogenic enzyme, comprising:

contacting a non-luminogenic enzyme with a liquid mixture comprising a luminogenic protein and a protected luminophore to form a composition; and detecting light produced from the composition.

63. (Withdrawn) The method of claim 62, wherein the protected luminophore is a modified coelenterazine;

wherein the enol group has been converted to an ester or an ether comprising an group that is removable by the non-luminogenic enzyme.

64. (Withdrawn) The method of claim 62, wherein the protected luminophore is a compound of formula (XII).

R⁸ is H, alkyl, heteroalkyl, or aryl;

R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

 R^{10} is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are independently enzyme-removable groups that are removable by the non-luminogenic enzyme.

65. (Withdrawn) The method of claim 62, wherein the protected luminophore is a compound of formula (XIII) or (XIV)

$$R^{11}O$$
 R^{7}
 R^{13}
 R^{12}
 R^{12}
 R^{13}
 R^{12}
 R^{13}
 R^{12}
 R^{13}
 R^{12}
 R^{13}

R⁸ is H, alkyl, heteroalkyl, or aryl;

R¹² and R¹³ are independently -H, -OH, alkyl, heteroalkyl, aryl, or -OR¹⁶; n is 0, 1, or 2; and

R¹¹, R¹⁴, and R¹⁶ are independently enzyme-removable groups that are removable by the non-luminogenic enzyme.

- 66. (Withdrawn) The kit of claim 34, further comprising DMSO or alcohol or a mixture thereof.
- 67. (Withdrawn) The kit of claim 38, further comprising DMSO or alcohol or a mixture thereof in the same container as the protected luminophore.
- 68. (Previously presented) The compound of claim 1, wherein R¹¹, R¹⁴, and R¹⁵ are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.
- 69. (Previously presented) The compound of claim 1, wherein R¹¹, R¹⁴, and R¹⁵ are independently selected from the group consisting of an alkyl group containing from 1-15 carbon atoms and a heteroalkyl group containing from 1-15 carbon atoms.
 - 70. (Currently amended) The compound of claim 1, wherein

- R¹¹, R¹⁴, and R¹⁵ are independently a heteroalkyl group containing from 1-20 carbon atoms, and wherein -OR¹¹, -OR¹⁴, and -OR¹⁵ are each independently comprising at least one of an ester group and or an ether group.
- 71. (Previously presented) The compound of claim 10, wherein R¹¹, R¹⁴, and R¹⁵ are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.
- 72. (Currently amended) The compound of claim 10, wherein R¹¹, R¹⁴, and R¹⁵ are independently a heteroalkyl group containing from 1-20 carbon atoms, and wherein -OR¹¹, -OR¹⁴, and -OR¹⁵ are each independently comprising at least one of an ester group and or an ether group.
- 73. (Previously presented) The compound of claim 16, wherein R¹¹, R¹⁴, and R¹⁶ are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.
- 74. (Currently amended) The compound of claim 16, wherein R¹¹, R¹⁴, and R¹⁶ are independently a heteroalkyl group containing from 1-20 carbon atoms, and wherein -OR¹¹, -OR¹⁴, and -OR¹⁵ are each independently comprising at least one of an ester group and or an ether group.
- 75. (Withdrawn) The method of claim 41, wherein R¹¹, R¹⁴, and R¹⁵ are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.
- 76. (Withdrawn) The method of claim 41, wherein R¹¹, R¹⁴, and R¹⁵ are independently a heteroalkyl group containing from 1-20 carbon atoms, and comprising at least one of an ester group and an ether group.

- 77. (Withdrawn) The method of claim 45, wherein R¹¹, R¹⁴, and R¹⁶ are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.
- 78. (Withdrawn) The method of claim 45, wherein R¹¹, R¹⁴, and R¹⁶ are independently a heteroalkyl group containing from 1-20 carbon atoms, and comprising at least one of an ester group and an ether group.